ON THE AMOEBAE PARASITIC IN THE HUMAN INTESTINE, WITH REMARKS ON THE LIFE-CYCLE OF *ENTAMOEBA COLI* IN CULTURES

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INTRODUCTION

The study of the parasitic Amoebae, although of the greatest importance, is one of considerable difficulty. Many investigators, in all parts of the world, have engaged in this study during the last twenty years with the most conflicting results. Consequently, to-day, the utmost confusion prevails as to the pathogenicity, morphology and culturability of the parasitic Amoebae of the human digestive tract, and at present nearly a dozen species are recorded from the human intestine alone. Further, nearly as many more species have been recorded from other organs of man.

Recently, while studying cultures of *Entamoeba coli*, as well as stools from patients being treated for dysentery at the Royal Southern Hospital, Liverpool, I have had occasion to examine the scattered literature on the subject. Before recording the preliminary results of my own studies it will be convenient to set forth a short critical review of the recent work on the parasitic intestinal Amoebae of man, as it seems to me that there has been a tendency to give undue prominence to Schaudinn's researches.

My work has been done in the Liverpool School of Tropical Medicine, under a grant from the Tropical Diseases Research Fund.

THE PARASITIC AMOEBAE OF THE INTESTINE OF MAN

Without adding to the complexity of the subject by a discussion of the history of the association of parasitic amoebae with human dysentery, we may at once give a list, with brief diagnoses, of the species of Amoeba recorded from the human intestine up to the end of 1910. We adopt the generic name *Entamoeba* of Casagrandi and Barbagallo (1895), and divide the parasites into those which are said to be pathogenic and those which are said to be non-pathogenic, beginning with the latter, thus:—

(A) Non-pathogenic forms.

1. *Entamoeba coli* (Lösch, 1875). Diameter, 12 to 25 μ, but variable.

No distinct ectoplasm apparent except at the beginning of pseudopodia formation. Endoplasm granular, filling up the body space when the organism is at rest. Nucleus large, sub-central, spherical, vesicular, containing much chromatin. Nucleus visible in the fresh state. Motility rather feeble.

Multiplication by binary fission or by schizogony, with formation of eight merozoites.

Encystment total and endogenous. Cysts 28 μ in diameter. Sporogony, after nuclear reduction and autogamy, with formation of eight amoebulae.

This parasite lives in the lumen of the large intestine, on the contents thereof; it is incapable of penetrating the mucosa. It may occur in the stools of healthy persons. It is usually considered to be non-pathogenic. Its possible pathogenicity is not above suspicion according to the researches of Billet (1907) and others. It can be cultivated in association with certain bacteria.

2. *E. tropicalis* (Lesage, 1908). This parasite is said to be non-pathogenic, and to occur in the intestine of man in the tropics. Though exhibiting a general resemblance to *E. coli*, and having a nucleus charged with chromatin, it is said to have a clearly distinguishable ectoplasm and to form small cysts (6 to 10 μ in diameter). The small size of the cyst is due to the amoeba having previously divided. Further the nucleus of the cyst is said to break

up into a variable number of daughter nuclei, so that from three to thirteen amoebulae may occur inside a cyst. Several varieties of this species are said to exist by Lesage. It is culturable in symbiosis with bacteria.

3. *E. hominis* (Walker, 1908). Diameter, 6 to 15 μ when at rest. Ectoplasm apparent only in the pseudopodia, endoplasm granular, nucleus circular. A single contractile vacuole present. Encystment total. Cysts small (4·6 to 7·7 μ). Sporulation frequent, spores spheroidal, measuring 0·3 to 0·8 μ.

Culturable with bacteria, but with difficulty. Original strain, now lost, from an autopsy in Boston City Hospital.

This species would appear to be closely allied to *E. tropicalis*.

(B) PATHOGENIC FORMS.

4. *Entamoeba histolytica* (Schaudinn, 1903), also described by Jürgens in 1902.

Diameter, 25 to 30 μ.* Ectoplasm clearly defined. Stout pseudopodia entirely composed of ectoplasm and capable of burrowing into the mucosa and sub-mucosa of the intestine. Nucleus variable in form, excentric and often lateral, poor in chromatin. Nucleus usually invisible in the fresh state. This parasite often ingests red blood corpuscles.

Multiplication by binary fission or by budding. Reproduction by exogenous encystment, giving rise peripherally to minute spores about 3 μ in diameter. The spores become encysted, and, according to Lesage, contain three nuclei.

It is stated that series cultures of this parasite, in association with bacteria, cannot be obtained, at any rate to retain their pathogenicity. Lesage (1907), however, claims to have cultured the parasite in leucocytic exudation from the peritoneum of infected guinea-pigs. The parasite has been found in cases of liver abscess and dysentery in Egypt, China and Japan.

5. *Entamoeba* sp., cultivated by Noc (1909), from cysts derived from liver abscess, from dysenteric stools and from the water supply of Saigon, Cochin China. Noc cultivated this amoeba in association with bacteria. It is, apparently, pathogenic, closely

* Hartmann (1909) gives a smaller size, 15-20 μ.

allied to *E. histolytica*, perhaps showing more marked *internal* budding (schizogony) than *E. histolytica* (judging by Noc's figures). It exhibits polymorphism, and may be a separate species, but is unnamed.

6. *E. tetragena* (Viereck, 1907), synonym *E. africana* (Hartmann).

Recorded from dysenteric cases in various parts of Africa, Brazil and India.

Diameter, 20 to 30 μ, according to Viereck.

Although the trophozoite of this amoeba bears a general resemblance to that of *E. coli*, yet it is said by Hartmann to possess a distinct ectoplasm which is only clearly visible when a pseudopodium is protruded. However, its granular endoplasm may contain ingested red blood corpuscles. There is a large round nucleus visible in the fresh state. Chromidial masses occur in the cytoplasm.

Multiplication proceeds by binary fission.

Sexual reproduction by endogenous encystment, which is preceded by nuclear division into two, reduction and then autogamy. The cysts contain four nuclei.

E. tetragena is pathogenic to man and to kittens, but the dysentery resulting is said to be more benign than that resulting from *E. histolytica*, and liver abscess is said to be rare.

E. tetragena is not culturable.

Hartmann (1909) stated that *E. histolytica* is a rare amoeba, and that in nearly all cases of amoebic dysentery the *E. tetragena* of Viereck is found.

Personally I have met with a case of chronic dysentery under treatment in Liverpool (probably infected in Nigeria), the parasite obtained from the stools being *E. tetragena.*

7. *E. phagocytoides* (Gauducheau, 1908).

This parasite was discovered in a case of dysentery at Hanoi, Indo-China. The amoeba is very small, 2 to 15 μ in diameter. It is active, and possesses a well-developed ectoplasm. It ingests bacteria and red blood corpuscles, while peculiar spirilla-like bodies are found in its cytoplasm.

It multiplies by binary and multiple fission.

Young cultural forms of this amoeba inoculated intravenously into a dog produced dysentery.

8. *E. minuta* (Elmassian, 1909). Found in association with *E. coli*, in a case of chronic dysentery in Paraguay.

It resembles *E. tetragena*, but is smaller, rarely exceeding 14 μ in diameter. No differentiation between ectoplasm and endoplasm. Nucleus invisible in fresh preparations, and when stained is richer in chromatin than that of *E. coli*.

Multiplication by schizogony into four merozoites.

Encystment total and endogenous, giving rise to cysts containing four nuclei, after nuclear reduction and autogamy.

9. *E. nipponica* (Koidzumi, 1909). Found in the motions of Japanese suffering from dysentery or from diarrhoea, in the former case in company with *E. histolytica*. It is also said to occur in healthy persons.

Diameter, 15 to 30 μ.

Clear distinction between ectoplasm and endoplasm. Pseudopodia are not spinose, but are lobopodia. Endoplasm vacuolated, and phagocytic for red blood corpuscles. The nucleus is well defined, and can be seen in the fresh condition; it is rich in chromatin, resembling that of *E. coli* and *E. tetragena*.

Multiplication by binary fission and by schizogony into six or eight merozoites.

Encystment total, and accompanied by formation of chromidia. The complete stages of sporogony have not been followed.

Experiments are necessary to determine the pathogenicity and culturability of this amoeba.

10. *E. undulans* (Castellani, 1905). Found, in company with other Protozoa, in the faeces of persons suffering from diarrhoea in Ceylon.

Diameter, 12 to 30 μ. There is an undulating membrane present, and long straight pseudopodia which appear rapidly, but only one pseudopodium is protruded at a time. Cytoplasm not differentiated into ectoplasm and endoplasm.

Obviously, further knowledge of this parasite is needed.

DIAGNOSIS TABLE

We may tabulate some of the various characteristics said to be diagnostic of the Entamoebae already mentioned thus:—

	Size	Ectoplasm and Endoplasm	Nucleus	Multiplication	Reproduction	Pathogenicity	Culturability
E. coli	12—25μ but variable	No distinct ectoplasm, except at beginning of pseudopodia formation	Round, vesicular, subcentral, with a karyosome. Visible in life	Binary fission. Schizogony—8 merozoites	Encystment total, endogenous, 8 spores in cyst	—	+
E. histolytica ...	25—30μ	Distinct. Endoplasm ingests red blood corpuscles. Burrowing pseudopodia	Small, excentric, poor in chromatin. Invisible in life	Binary fission. Budding	Small exogenous spores	+	—
E. tetragena.....	20—30μ	Ectoplasm distinct when pseudopodia formed. Endoplasm ingests red blood corpuscles	Round. Visible fresh	Binary fission	Encystment total, endogenous. Cysts contain 4 nuclei	+	—
E. tropicalis.....		Distinct	Round, rich in chromatin	Binary and multiple fission (?)	Encystment total, endogenous, numerous small spores in small cyst	—	+
E. hominis	6—15μ	Ectoplasm apparent only in the pseudopodia. Single contractile vacuole present	Round	'By division and by sporulation'	Cysts 4·6—7·7μ	?	+
E. phagocytoides	2—15μ	Ectoplasm well developed. Active. Phagocytic		Binary fission. Schizogony		+	+
E. minuta	14μ	Not distinct	Round, rich in chromatin. Invisible in life	Schizogony—4 merozoites	Encystment total, endogenous. Cysts with 4 nuclei	+	—
E. nipponica ...	15—30μ	Distinct	Round, rich in chromatin. Visible fresh	Binary fission. Schizogony —6 to 8 merozoites	Encystment total. Incompletely known	?	?

NOTE.—*E. histolytica* differs from all the others in that its encystment is exogenous, not total and endogenous as in the other cases where the sporogony is known. From this table it will be seen at once how slight are many of the differences between the so-called species of *Entamoeba* in the human intestine.

SOME CRITICAL REMARKS ON THE VARIOUS INTESTINAL AMOEBAE OF MAN

It is now usually recognised, since the experimental researches of Schaudinn (1903) and others, that amoebae of two kinds may occur in the human digestive tract, namely, pathogenic ones and others which are non-pathogenic. To the latter class belong *Entamoeba coli* and *E. tropicalis*. In the former class must be placed *Entamoeba histolytica* and *E. tetragena*. Further, the non-pathogenic forms are culturable with symbiotic bacteria, while the pathogenic ones are not so culturable, or doubtfully so.

Musgrave and Clegg (1904) were the pioneers of successful modern cultural methods as applied to amoebae. These distinguished workers, however, suggest that 'all amoebas [in the intestine] are, or may become pathogenic,' and state that 'amoebas cultivated from various sources, including the dysenteric intestine, the Manila water-supply, lettuce, etc., have proved pathogenic under certain conditions, which reverses the view held of some of those formerly considered harmless.'

The discrepancy between Musgrave and Clegg's results and those of Schaudinn is usually ascribed to impurity of cultures, due to the presence, unnoticed, of the small cysts of *E. histolytica* in the cultures of the Philippine observers. Lesage (1908) considers that Musgrave and Clegg cultured chiefly *E. tropicalis*, while Werner (1908) thinks that they had *Amoeba limax* in their cultures, and he similarly criticises Walker. But we must still carefully consider Musgrave and Clegg's results, especially when we remember that the possible pathogenicity of *E. coli* is not above suspicion (cf. Billet, 1907), and that quite recently (November, 1909) Elmassian asks how we are to interpret the occurrence of *E. histolytica* in non-dysenteric natives in Asia. Obviously, many further researches are needed.

Regarding the large number of species of parasitic amoebae recorded from the human intestine, I think that few of the so-called species are really good ones. With respect to plurality of species, we must carefully consider the phenomenon of *polymorphism*, a phenomenon markedly exhibited by amoebae. Many of the species are apparently only separated by slight morphological differences,

such as in the distinctness or otherwise of the ectoplasm from the endoplasm, in the structure of the nucleus, or even in size. Such differentiation is unsatisfactory, as must be evident to any investigator who has worked for any length of time on *one* species of organism, who has fixed and stained it by various methods from day to day, and compared the results obtained. Morphological variation, then, must not be overlooked when separating species. Both Musgrave and Clegg (1906) and Noc (1909) have recorded the occurrence of variability in morphological characters of amoebae in cultures started from a single, isolated cyst.

Certain observers, again, have cultivated species of amoebae, and then omitted to test the pathogenicity or otherwise of the cultural forms by experiments on animals. Further, the life-cycles of cultural amoebae must be carefully examined and compared with the natural forms, a point which appears to have been largely overlooked.

It seems to me that the methods of reproduction supply the most valid grounds on which to base species differences, taken in conjunction with possible pathogenicity. We then have three fairly well recognised species of amoebae parasitic in the human intestine, namely:—

(1) *Entamoeba coli*, with its varieties *E. tropicalis* and possibly *E. hominis*. These are apparently non-pathogenic. The encystment is total and endogenous.

(2) *E. histolytica*, the pathogenic agent in certain cases of dysentery and liver abscess recorded from Egypt and China, and perhaps from Europe. The encystment is not total but exogenous, and minute spores are produced. The organism has been best studied by Schaudinn (1903), Craig (1908) and Hartmann (1909).

(3) *E. tetragena*, the pathogenic agent of dysentery in cases recorded from various parts of Africa, Brazil and India. The encystment is total and endogenous. The organism has been studied by Viereck (1907) and Hartmann (1908).

Probably *E. minuta* is merely a variety of *E. tetragena*, while *E. nipponica* seems to belong, as a variety, either to *E. coli* or to

E. tetragena. The position of *E. phagocytoides* is unsatisfactory until its sporogony has been investigated.

Gauducheau (1909) states that at a later stage of the culture the organisms (*E. phagocytoides*) are difficult to keep alive, and then are only about 1 μ in diameter. Brown (1910) considers that Gauducheau's organism clearly shows affinities with *E. histolytica*, like Noc's entamoeba.

Morphologically *E. coli* and *E. tetragena* are somewhat alike and form endogenous cysts, though the daughter forms within the cyst are eight and four respectively. It is interesting to note that Viereck (1907) first thought that *E. tetragena* was a variety of *E. coli*. Our knowledge of *E. tetragena* is not yet quite complete.

PRELIMINARY NOTE ON THE LIFE-HISTORY OF *ENTAMOEBA COLI* AS SEEN IN CULTURES

Two separate cultures of *Entamoeba coli* have been examined. They were derived from dysenteric cases from Manila, and have been maintained on Musgrave and Clegg's medium (at 20° to 25° C.) by sub-inoculations for some three years. I have much pleasure in thanking Dr. Stephens for the material.

The life-cycle of the parasite in cultures has been studied, a point which does not appear to have been fully recorded in previous literature.

The results, not yet complete, may be summarised as follows. On Musgrave and Clegg's medium the amoebae leave their cysts after rupturing them, and some discarded empty cysts may be found in preparations. Sometimes, however, the encysted parasite first appears to swell up or grow, the cyst wall gradually becoming thinner meanwhile until it appears ultimately to be absorbed. Small vacuoles may occur in the cyst. The amoeba when first free usually contains one or two small vacuoles which after combining slightly enlarge and travel to the periphery. This vacuole aids in the protrusion of the first pseudopodium. The pseudopodia are composed chiefly of ectoplasm, though endoplasm flows in to some extent later. The greater part of the body of the amoeba is composed of granular endoplasm, and some of the larger granules may stain metachromatically with methylene blue *intra vitam*. The

amoeba now feeds, grows and moves about in a restricted area which is often approximately circular. The nucleus of the amoeba is round and vesicular with a central karyosome, and is clearly visible in life. There is a clear area in the endoplasm around the nucleus. The parasite at this stage may be called a trophozoite, in preference to the term 'vegetative' stage which is so often used. The amoeba divides by binary fission with nuclear promitosis. in which the karyosome plays an important part. The parasite also divides, occasionally, by schizogony, forming eight merozoites.

On the culture-media which I have been using, the amoebae begin to encyst in about four days, the cyst wall of each being formed by differentiation at the periphery of the now rounded amoeba. The encystment is total. The cyst at first contains a centrally-placed nucleus, with a karyosome. Inside some of the cysts division occurs, and eight daughter forms are produced. The cytological details of sporogony are now being studied, and will be published later. When all the amoebae in a culture have encysted and remained in that condition for some time a new culture must be prepared. The cultures with which I have been working are renewed about every fortnight or three weeks.

I have tried the action on *Entamoeba coli* of some of Dr. H. C. Ross's 'auxetics'—substances capable of inducing division in living cells. I wish to thank Drs. H. C. Ross and J. W. Cropper for providing me with some of these substances. Many of these auxetics occur naturally in the body, and my attention has been especially directed to some which are found in the intestines, such as tyrosin, leucin and skatol.

These substances are best used in a jelly with agar, sodium chloride and alkali (sodium bicarbonate), forming a slightly alkaline culture medium. When such a medium, containing about 0·2 per cent. of tyrosin, is inoculated with cysts of *E. coli* obtained from a culture on Musgrave and Clegg's medium, the period of the life-cycle is shortened, and the amoebae on the culture reproduce for several generations. I have a culture which has already gone through five generations. Somewhat similar results occur on a culture-medium containing a similar quantity of leucin. Unfortunately the 'growth' does not increase much, for there is

apparently rapid death of some of the amoebae, probably resulting from insufficient food-supply in the media.

One interesting and novel result on tyrosin-containing media, compared with cultures on Musgrave and Clegg's medium, is that a complete life-cycle of *E. coli* is passed through in about three days (at 20° to 25° C.), when all the amoebae of a given generation have encysted. Then a large number of the cysts produce eight daughter forms inside them, and the amoebulae come out of the cysts and start a new generation on the same medium. I have seen these phenomena continued through five generations, whereas on Musgrave and Clegg's medium only one generation of amoebae is usually produced, and few of the cysts give rise to eight daughter forms. Further, binary fission of amoebae occurs on a tyrosin-containing medium more frequently than on a Musgrave and Clegg medium. The process of binary fission involves a primitive mitosis (or promitosis) of the nucleus, caps of chromatin derived from the karyosome being formed at the ends of the rudimentary spindle. Stages of schizogony have also been seen.

Skatol added to a preparation containing free *E. coli* rapidly induces encystment. This is of interest since skatol occurs naturally in the hinder part of the digestive tract and in the faeces.

Auxetics such as supra-renal extract and metaphenylenediamine also apparently induce division. The binary fission in such cases is of the nature of unequal promitosis, probably due to the rapidity with which it is induced.

These researches are being continued, and I hope to publish a more detailed and illustrated account later, in which the cultural forms will be compared with the amoebae in their natural habitat.

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